THE INSTITUTE FOR CANCER RESEARCH
and
THE LANKENAU HOSPITAL RESEARCH INSTITUTE
7701 Burholme Avenue
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November 18, 1954

Professor Joshua Lederberg
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Dear Joshua:

We were delighted to hear from you that you will participate in the Conference on Ascites Tumors in New York next May 19th and 20th. Not only was there no trouble in justifying your appearance, but the meeting would have to be considered very incomplete without discussion of our general problems by a creative worker in the field of microbiological genetics. As you say, some of the parallelisms which have been drawn between mammalian tissue genetics and bacterial population problems have been rather sloppy and far fetched. We are counting on you to dispel the fog.

It will probably be possible, somewhere in the program, to draw out Lettré on the subject of mitochondrial recombination with granule-free cells. We have seen considerable evidence in our daily handling of various ascites tumors for incorporation of particulate matter by various types of ascites cells, but have not done anything about it. Off-hand, I am not adverse to accepting Lettré's story, which however, should be repeated more critically. It belongs in the same chapter as "Incorporation of Chromatin Fraction by Cells". Although, I am disinclined (because of my personal experience with rather high-take percentages after inoculation of single cells) toward believing with my friends, Paschkis and Cantarow, in transduction by chromatin fraction, I do not feel that George Klein's genetic evidence, published in CANCER RESEARCH in 1952, settles the issue entirely.

Your idea, suggested to George Klein, of using a genetic dependence or resistance marker in addition to histocompatibility, appeals to me very much, and some one should do this. The other day I leaned over backwards to think of all the reasons for continuing with a little further work on chromatin fraction, and I put these ideas down rather informally for Dr. Paschkis. A copy of this discussion is enclosed. You probably won't like it.

Under separate cover two brief items of recent vintage are being sent for your reprint files.

With sincere good wishes and regards to Mrs. Lederberg,

Yours ever,

Ted

Theodore S. Hauschka

TSH/ems
Enclosure

Discussion of Klein's results after injection of chromatin-fraction of two mouse lymphomas into F_1 hybrids

(See Cancer Research, 12:589-590, 1952)

A. Points in favor of Klein's argument that chromatin-fraction was probably contaminated with 6C3HED or DBA lymphoma cells:

(1) Rapidity of appearance of tumors (10-27 days after inoculation of chromatin-fraction). This time element is comparable with Hauschka's observations after i.p. inoculation of 20-30 6C3HED or DBA cells per mouse.

(2) Transplantability of tumors "induced" in F_1 into parent type of tumor origin.

B. Points weakening Klein's argument.

(1) The tumors which appeared in F_1 after the chromatin-fraction was injected were (with a single exception) all solid tumors, while Hauschka obtained 100% ascites after inoculation of 20-30 intact viable cells.

(2) Exceptions are known to the genetic "rule" that F_1 tissue is transplantable only into comparable F_1 hybrid hosts (See Little's review in "Genetics in the 20th Century").

(3) Failure to detect intact cells or intact nuclei in chromatin-fraction.

C. Information not included in Klein's experimental scheme.

(1) Results after known small number of intact lymphoma cells is put into F_1 mice. Are the resulting tumors solid or ascites? How many cells are needed to produce 100% takes in F_1 mice within the time limits of the chromatin-fraction result? Hauschka has found F_1 hosts more resistant to low cell dosages than pure C3H or DBA mice.

(2) Results after tumor chromatin-fraction is put directly into susceptible strain in which tumor originated. Are the resulting growths solid or ascites?

D. Klein's experiments, while providing seemingly strong genetic evidence for the contamination of tumor chromatin-fraction with a few viable cells are not, therefore, entirely conclusive.

The sequence of events through which a normal F_1 lymphocyte might be converted into a malignant one, which then becomes transplantable into the parental genotype providing the chromatin-fraction, involves:

(1) Incorporation of functional chromatin elements into F_1 lymphocytes. (Uptake of particulate material by mammalian cells of several types, both in vitro and in vivo is a well-established phenomenon. See, for instance, Lettre's recent results with Ehrlich ascites cells.)

(2) Change of at least one cell, but judging from the short latent period several normal F_1 lymphocytes into neoplastic elements. This would seem to presuppose that the malignant change is inherent in a portion of the chromosomal material of the chromatin-fraction or in a contaminating cytoplasmic entity. If functional chromosomal elements are involved, it must be assumed further that - after getting into the cell - these specific genetic entities are incorporated into the nucleus and equally distributed among daughter cells in subsequent mitoses.

(3) Uptake of functional lymphosarcoma chromatin-fraction by normal F_1 lymphocytes, which have thereby become neoplastic, would also have to neutralize the iso-antigenic character of those histocompatibility factors which entered into the susceptible F_1 mouse through the non-susceptible parent strain, the latter being 100% refractory to the tumor furnishing the chromatin-fraction. Granting the mechanical premise of chromatin uptake, the possibility of antigenic modification by specific iso-antigenic entities in the chromatin-fraction is compatible with our demonstration (Hauschka, T.S., and Levan, A. Inverse relationship between chromosome ploidy and host-specificity of sixteen transplantable tumors. Exper. Cell Research, 4:457-467, 1953) of a consistent inter-relationship between aneuploidy and decreased transplantation specificity. Incorporation of antigen-carrying chromatin-fraction could conceivably make an F_1 lymphocyte aneuploid with respect to the specific gene-action which controls antigenic end-products and, thus, would tend to favor transplantability beyond the limits of classical genetic expectation, at least into the susceptible parental genotype.

(4) The following cytologic data may be relevant here (see Levan, A., and Hauschka, T. S. Nuclear fragmentation--a normal feature of the mitotic cycle of lymphosarcoma cells. Hereditas, 39:137-148, 1953): Although nuclear fragmentation and lobation is a frequent phenomenon in some mouse lymphosarcomas, a mechanism exists for the reconstitution of a single normal metaphase plate, after the separate micronuclei have undergone synchronous prophases. Anaphase is normal and telophase culminates again in nuclear lobation and fragmentation. Bits of chromatin material with functional kinetochores, which manage to get into an F_1 lymphocyte, might behave like the above micronuclei and become part of the functional genome of the cell, rendering it malignant and increasing its transplantation range.

(5) Obviously, a number of rather labored assumptions are needed to circumvent Klein's classical genetic argument. It should be remembered, however, that histocompatibility genetics is no longer the watertight aggregate of laws it was even three years ago. Difficulties and the need for new interpretation were introduced into the field by:

(a) the influence of heteroploidy on the antigenic specificity of grafts (Hauschka);

(b) the F_1 adaptation phenomenon (Barrett et al., Hauschka);

(c) the in utero conditioning of mice to accept, in adult life, persistent skin-grafts to which they are genetically refractory (Billingham et al.);

(d) antigenic conditioning of non-susceptible hosts by cell-free enhancing substance (Kaliss, Snell et al.).

It, therefore, appears equally probable that specific chromatin-fraction might so alter F_1 cells, that they become genetically compatible with the donor-type of the chromatin.

E. Suggestion for a possibly crucial experiment.

Insert tumor-chromatin-fraction into new type of Algire disc chamber and sew into the peritoneal cavity of animal genetically compatible with the tumor from which the fraction was prepared. Since disc does not (usually) permit cells to pass, no tumor should arise inside the chamber if chromatin-fraction contains no cells.

If the fraction is, on the other hand, contaminated with viable cells, then tumor should start to grow within the chamber after a latent period.

Controls could include:

(1) Small known number of viable malignant cells added to chromatin-fraction inside disc.

(2) A few neoplastic cells only, inside disc.

(3) A mixture of normal lymphocytes plus malignant chromatin-fraction inside disc.

Theodore S. Hauschka
November 11, 1954